Metrology and Standards Needs for Gene Expression Technologies: Universal RNA Standards

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(www.cstl.nist.gov/biotech)
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Stanford University

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National Institute of Standards and Technology
Technology Administration, U.S. Department of Commerce

Overview

- Introduction to NIST
- Biotechnology at NIST
- Fluorescence & GenModOrg Examples
- Measurements & Standards: Making Choices
- Workshop Goals

NIST is a non-regulatory federal agency within the Technology Administration of the U.S. Department of Commerce. NIST's primary mission is to **promote economic growth by working with industry to develop and apply technology, measurements, and standards**.

NIST carries out its mission through a portfolio of four programs:

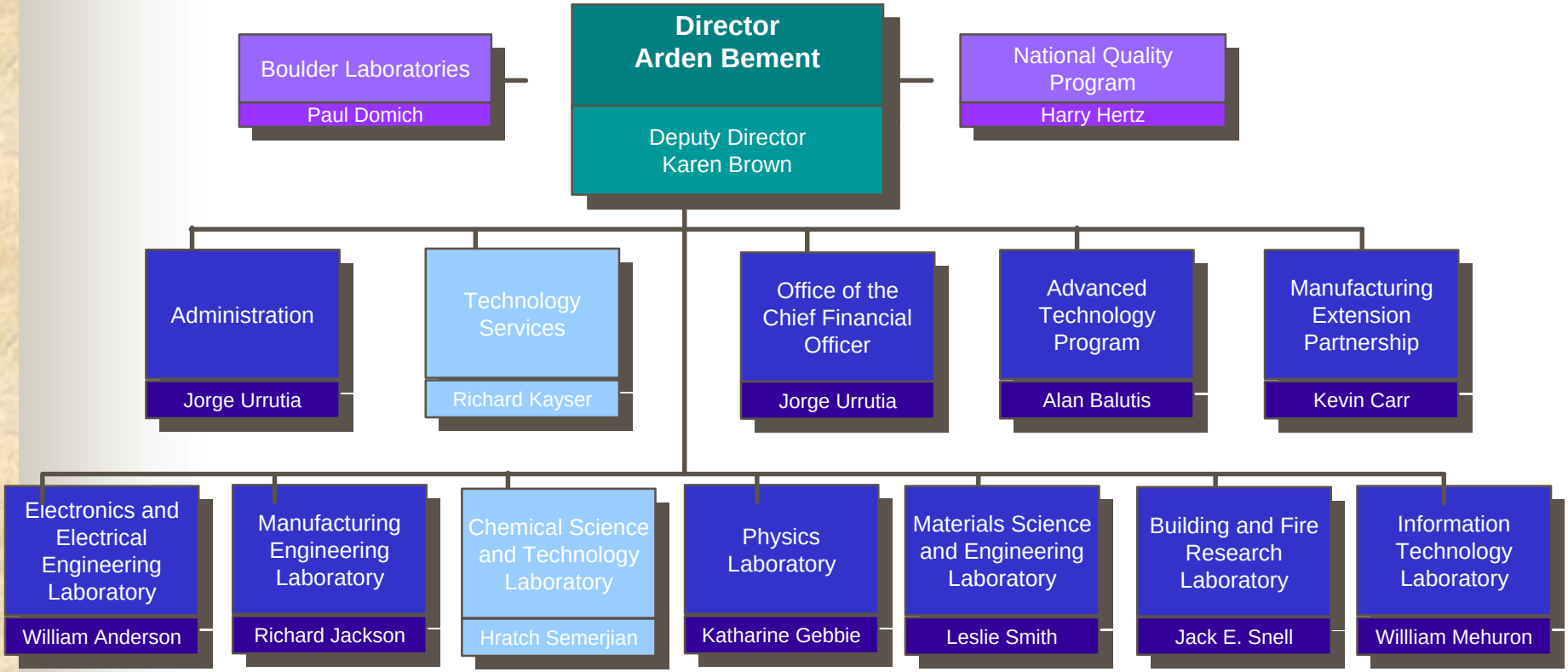
Measurement and Standards Program
planned and conducted in cooperation with industry and focused on infrastructural technologies

Advanced Technology Program
cost-shared, competitive awards to industry for development of high-risk, pre-product, enabling technologies

Manufacturing Extension Partnership Program
a nationwide network of extension centers that provides hands-on technical assistance to smaller manufacturers

National Quality Program
an outreach program recognizing quality improvement by U.S. manufacturing and service companies

National Institute of Standards and Technology



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Vincent Vilker, Chief

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Biomolecular Materials

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Gas Metrology and Classical Methods
Chemical Sensing and Automation Technology
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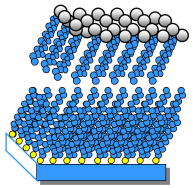
CSTL FY02 Programs

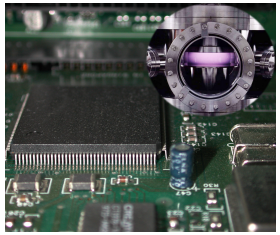
Transportation

Biomaterials



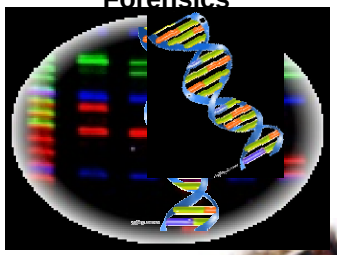
Energy Systems

Microelectronics



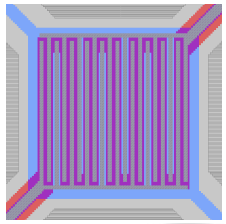
Environmental Technologies

Forensics



Biotechnology

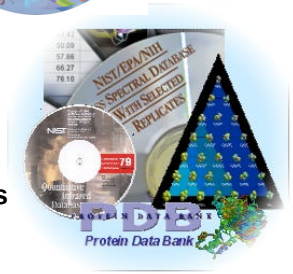
Nanotechnology



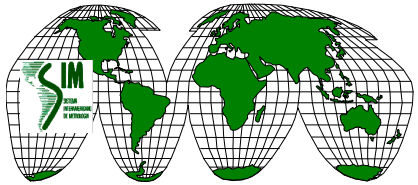
Healthcare

Food and Nutrition

Chemical and Allied Products



Data and Informatics



International Measurement Standards

Industrial and Analytical Instrument Services

March 28-29, 2003
Metrology for Gene Expression RNA Standards

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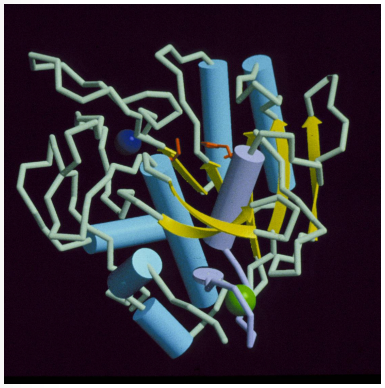
NIST Biotechnology Division

- **DNA Technologies**
  - Human Identification and Forensics
  - DNA Diagnostics
  - DNA Damage and Repair
  - DNA Quantification & GMOs
- **Bioprocess Engineering**
  - Biocatalytic Systems
  - Biothermodynamics
  - Biospectroscopy
  - Bioseparations



- **Biomolecular Materials**
  - Lipid Membranes and Membrane Proteins
  - Nanotechnology
  - Biosensors
  - Tissue Engineering
- **Structural Biology & Computational Biology**
  - Macromolecular Structure - X-ray & NMR
  - Macromolecular Functional Characterization
  - Modeling and Computational Chemistry
  - Structural Biology Databases
  - Bioinformatics

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Program Guidance

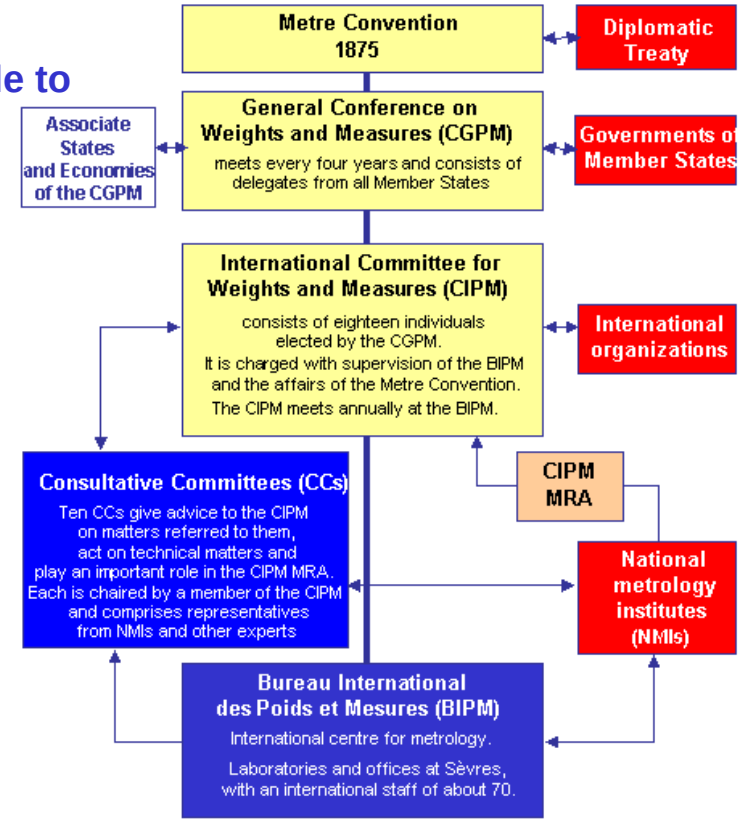
- *Industry* – BIO, direct interactions, …
- *Scientific Community* – ACS, ASBMB, ABRF, IUPAC, FASEB, ACA, ASM, …
- *Government Agencies* –**NRC**, FDA, CDC, NIH, NIJ, DOD, USDA, OSTP/BRWG …
- *Standards Activities* – ASTM, NCCLS, **CCQM/BIPM** …
- Workshops – Fluorescence, Crystallography, CASP, AFM, Proteomics, Biotech Grains, Microarrays…

Growing International Outlook

Provides the framework within which the international measurement system is maintained and made available to the whole world for:

- **national and international trade**
- **manufacturing**
- **human health and safety**
- **the protection of the environment, and**
- **all aspects of science and engineering**

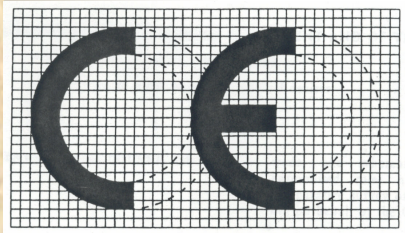
METRE CONVENTION



New Regulatory Requirement: EU IVD Directive

A New Driver:
EU IVD Directive to go into effect 2003

- **Worldwide *in vitro* diagnostic device market is ~$20B;**
- **>60% of European market is supplied by U.S.**



Stated Purpose of Directive

- **Eliminate trade barriers *within Europe* by ensuring access to the entire EU market with one single product approval (CE Mark)**

Essential Requirements

- **IVD Calibrators and/or control materials must be traceable to *"standards of a higher order"***
  - **nationally/internationally recognized *certified reference materials***

US IVD Manufacturers have requested that NIST develop internationally recognized reference methods and SRMs to meet the traceability requirement

Scheduled Implementation

- **First IVD product with CE Mark may be placed from June 2000 onwards**
- **All *new* IVD products *must* have mark by December 2003 (may be delayed)**
- **Existing IVD products may be sold without the CE mark until December 2005**

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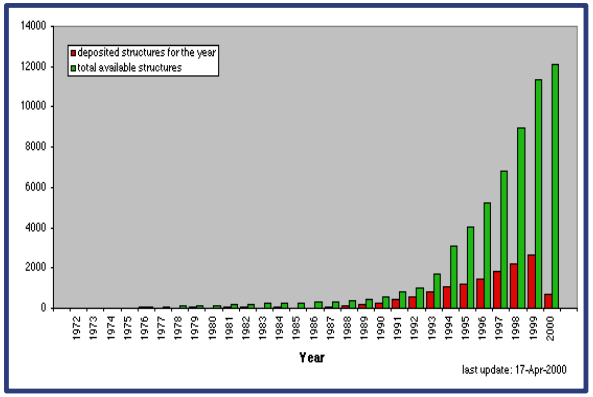
NIST Biotechnology Products

STANDARD REFERENCE MATERIALS

- Human Identification and Forensics
  - –DNA Profiling (SRM1950, SRM 1951a,b)
  - –Mitochondrial DNA (SRM 1956a, b)
- DNA Diagnostics (p53 & HER2 SRMs in prep)
- DNA Damage & Repair (SRM in prep)
- Fluorescence (SRM 1932, in prep 1933)
- Peptides (SRM in prep)



DATA ACTIVITIES

RCSB – Protein Data Bank (PDB)

BioMacromolecule Crystallization Database (SRD 21)

Short Tandem Repeat DNA Database

Thermodynamics of Enzyme-Catalyzed Reaction (SRD 74)

Future Emphasis Areas

- Proteomics
- Tissue Engineering
- Microbial Forensics
- DNA Diagnostics
- Gene Expression
- Genetically Modified Organisms

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Fluorescence

- **FDA & CDC brought attention to need for flow cytometry (fluorescence) standards**
- **NIST Fluorescence Workshops Jan ‘98, Nov ‘98, June ‘00 – Industry, Academia, Government Agencies, NMI’s**
- **Results of workshops indicated critical need for fluorescence signal standardization required for quantifying biological information (e.g., genomic data), and development of high-throughput screening methods**
- **Certification of first NIST solution fluorescence SRM 1932 in Sep ‘02**

SRMS for Flow Cytometry

•SRM 1932 Fluorescein Solution

- **Certified for concentration and purity**
- **Used for fluorometer calibration**
- **Enables a NIST-traceable MESF fluorescence intensity scale to be established**

(MESF = molecules of equivalent soluble fluorophore)

- SRM 1933 Fluorescein-Labeled Microbead Suspension (*under development)*
  - **To be used for flow cytometer calibration (MESF intensity)**
  - **Will help meet the growing need for quantifying analyte per cell levels**

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Genetically Modified Organisms (GMOs)
aka Biotech Grains

Needs Assessment

NIST sponsored workshop held in December 2001 with representation from the EU, Asia-Pacific Rim, and all five subregions within the Americas (SIM), to discuss:

- Regulatory differences
- Existing measurements (genetic modification, expressed proteins)
- Gap analysis
- Plan of action

Current Activities

- Work with USDA GIPSA to develop SRMs to support U.S. Grain exports; critically evaluate and employ existing technologies
- Investigate the use of LC/ESI-MS to measure proteins in GMO reference materials (BCR CRMs); use results to validate ELISA test kits currently used to determine whether genetically modified protein present.
- Use LC/ESI-MS results to help "train" near IR method for high throughput screening for detection of GMO grains in large samples

Measurements & Standards: Priority Setting

- **Magnitude and immediacy of need**
  - ➔ There is a problem.
- **Ability to make a difference; nature and size of anticipated impact.**
  - ➔ **NIST response** (such as a Reference Method, Appropriate Publication, SRM, etc.) **can provide a solution.**
- **Ability to respond in a timely fashion**
  - ➔ Adequate funding, appropriate staff, and facilities can be made available.

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Measurements & Standards: An Example (Clinical Diagnostic Markers)

Problem Magnitude and Scope:

U.S. Spends ~ $1.1 trillion on Health Care)

–~10-15% of this amount is associated with measurement ($140B)

–Non-diagnostic measurements cost ~$40B

German Health report 1998 (www.gbe-bund.de):

–Costs of repeat measurements amounts to 1.5 B US$ annually in Germany

New Driver: EU IVD Directive to go into effect 2003

Worldwide in vitro diagnostic device market is ~$20B;
>60% of European market is supplied by U.S. based companies

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Measurements & Standards:
Value Assignment of Reference Materials

Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements

	Certified Value	Reference Value	Information Value
1. **Certification at NIST Using a Primary Method with Confirmation by Other Method(s)**	✓		
2. **Certification at NIST Using Two Independent Critically-Evaluated Methods**	✓	✓	
3. Certification/Value-Assignment **Using One Method at NIST** and Different Methods by Outside Collaborating Laboratories	✓	✓	
4. Value-Assignment Based On Measurements by Two or More Laboratories Using Different Methods in Collaboration with NIST		✓	✓
5. Value-Assignment Based on a Method-Specific Protocol		✓	✓
6. Value-Assignment Based on NIST Measurements Using a Single Method or Measurements by an Outside Collaborating Laboratory Using a Single Method		✓	✓
7. Value-Assignment Based on Selected Data from Interlaboratory Studies		✓	✓

Gene Expression Technologies

Measurements and Standards

Oct 15, 2002 --- NIST Meeting on Metrology and Standards Needs for Gene Expression Technologies (Device Makers, Technology Users, Regulators)

Dec 10, 2002 – NIST/Industry Workshop on Standards Needs for Microarrays (Device Makers, Reagent Makers, Technology Users, Regulators)

March 28-29, 2003 – NIST/Industry Workshop on Universal RNA Standards (Device Makers, Reagent Makers, Technology Users, Regulators)

Gene Expression Technology

Gene Expression as a Measurement System

If the goal is to characterize gene expression technologies as measurement systems

Then we need to define Metrology Figures of Merit

- Sensitivity (how much RNA, ……..)
- Specificity (relevant features of RNA, …….)
- Precision
- Systematic Errors (Biases)
  - **Sources of RNA**
  - **Detection Platforms**
- ???

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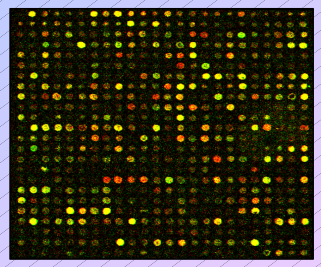
Gene Expression Technologies

Microarray Standards

Microarray Readers

- DNA & Protein Chips



- ***What's Needed***
  - **Fluorescence intensity standards at specific colors**
- ***What We Are Doing***
  - **Working with array reader manufacturers (e.g. Affymetrix, Agilent, Axon, Perkin Elmer) to determine the specs for effective standards**
  - **Developing fluorescent materials that possess desired specs**
- ***Impact***
  - **Enable the quantitation of instrument performance**
  - **Improve assay reproducibility and accuracy**
  - **Reduce cost**

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Workshop Goals

- **Identify key measurement issues facing technology**
  - *short- and long-term*
- **Will all platforms (microarray only?) benefit from identified issues**
- **Are identified issues NIST *(applied biology)* or NIH *(discovery biology)* related**
  - *differences*
- **Can issues be resolved without NIST**
  - *why & why not (pros & cons)*
- **Should NIST resolve these issues**
  - *define role NIST should take*
  - *identify partnerships with NIST to address issues*
- **Will microarrays require FDA approval for P.O.C. and diagnostic market**
  - *identify industry (research and diagnostic) consequences of not addressing issues of metrology*

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Thank you ……

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Krishna Ghosh, Agilent Technologies

Catherine O'Connell, NIST

Janet Warrington, Affymetrix, Inc

and

Ron Davis, Stanford